2.—Copper Poisoning in Sheep in Western Australia

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Investigations were made to ascertain the cause of high-copper status of sheep in the Wiluna area of Western Australia. It was concluded that this was due mainly to the ingestion of plants naturally high in copper.

An occurrence of copper poisoning in sheep in the Toodyay area was also investigated. Here the copper content of pastures generally was not high. Although the factors responsible for the high copper status of sheep were not determined, histological evidence suggested that this was associated with a hepatotoxic principle of plant or fungal origin.

Introduction

During investigations relating to the deficiency of copper in stock in Western Australia and in the course of routine laboratory diagnosis, we have encountered numerous cases of copper poisoning in sheep. In some instances the cause has been due to an over-generous use of copper-containing licks or fertilizers, often to both. In other cases the poisoning has been associated with lupinosis when the damaged liver accumulates excessive amounts of copper, particularly if copper supplements have been fed. Investigations into this disease and the significance of the storage of heavy metals in the liver are being continued by the Department of Agriculture at the present time.

In eastern Australia, copper poisoning is commonly associated with plants containing pyrrolizidine alkaloids, particularly heliotrope *(Heliotropium europaeum)* and "Pattersons Curse" *(Echium plantagineum)* (Bull 1961; St. George-Grambauer and Rac 1962). The first plant is rare in southern Western Australia and no cases of copper poisoning due to either plant have been reported.

The purpose of the present paper is to record the results of detailed investigations of the cause of the high copper status of sheep at Wiluna in the North Eastern Goldfields pastoral region, and at Toodyay.

The High Copper Status of Sheep in the Wiluna Area

In 1951 one of us (H.W.B.) visited the Eastern Goldfields area of Western Australia to enquire into possible causes of sheep losses in the 1950-51 drought. Some pastoralists had considered that the losses were unduly severe and could not be attributed solely to the effects of drought. On some stations it was reported that sheep had lost their appetite for top feed which should have been adequate for their requirements. As a result of these observations it was decided to investigate the remote possibility that cobalt deficiency was responsible for this reported anorexia. During the analysis for cobalt content it was noted that the livers contained large amounts of copper. Shortly afterwards chronic copper poisoning was diagnosed at "Albion Downs" Station. The present investigation was then carried out to ascertain the reasons for the high copper status of sheep at Wiluna and the area of country affected.

Because of the remoteness of the region and of the large areas of the properties concerned, the scope of the investigation was restricted to the determination of copper in the livers of sheep from nine station properties, to the analysis of herbage from three properties where sheep showed high copper status and to the determination of copper in well waters on one property. Histological examination was made on many of the liver samples to check for the possible effects of hepatotoxic plants.

Materials and Methods

At "Albion Downs" Station, every species likely to be eaten by sheep was collected in the 1953 sampling. The sampling was much less comprehensive at later dates and on other stations.

Soil contamination was avoided in the collection of samples but in some short and semi-prostrate species it was not possible to avoid this entirely. In these cases an iron determination was done to obtain some indication of soil contamination.

As levels of molybdenum, manganese and inorganic sulphate are known to influence copper metabolism in sheep, determinations of these constituents were made on many of the herbage samples. Copper, molybdenum and inorganic sulphate were determined as described previously (Beck 1962), manganese by the periodate method (Willard and Greathouse 1917) and iron by the thioglycollic acid method (Mayer and Bradshaw 1951).

Except for one sheep which died from copper-poisoning, all liver samples were from healthy sheep killed for rations. The livers were preserved in copper-free alcohol or alcohol-formalin mixture.

The analysis of all samples is reported on the dry-matter basis. No correction was made for the fat content of livers, but no obvious fat was noted in any of the samples.

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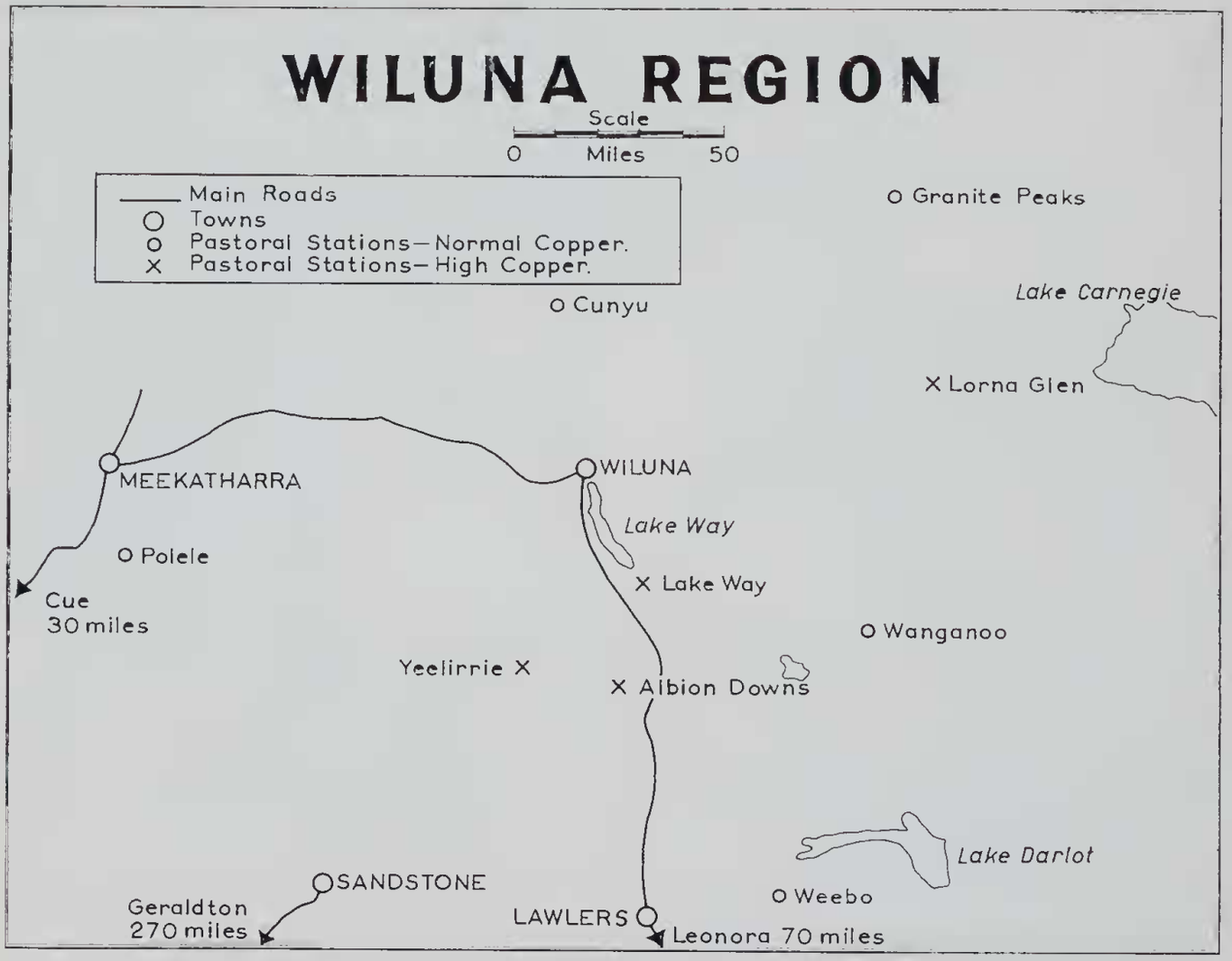

Fig. 1.

Results

An examination of well waters was made on "Albion Downs" Station in 1953 but the copper levels were less than 0.04 mg per litre and would not be responsible for the high levels of copper in liver.

The analytical data for liver samples and for herbage material are set out in Tables I and II respectively. The livers of normal Western Australian sheep contain 50 to 400 p.p.m. copper and as sheep with levels over 1000 p.p.m. are liable to develop copper poisoning under conditions of stress, the percentage of such samples is indicated in Table I. The geographical distribution of properties is shown in the accompanying map.

With *Goodenia Mueckeana* and *Eremophila leucophylla* there was little difference in copper content at the different times of sampling, but in all other species the levels in the 1953 samples were appreciably higher. There was no obvious difference in other constituents of species at different sampling times or on the different properties.

A number of livers from ration sheep have been examined histologically for evidence of ingestion of hepatotoxic plants. Some of the high-copper samples showed small areas of megalocytosis but there was no significant liver damage in any of the specimens.

Prior to the outbreak of copper poisoning in 1952 it had been noted that the affected sheep had been grazing heavily on *Goodenia eremophila*. This species did not reappear in appreciable amounts until 1959 when a sample was forwarded to the C.S.I.R.O. Chemical Research Laboratories, Melbourne, for alkaloid determination. The analysis showed a very low alkaloid content (0.015 per cent. tertiary base, on assumed molecular weight of 300; N oxides were absent) which would be unlikely to cause any liver damage.

Discussion

From the data obtained it would seem that the occurrence of high copper status in merino sheep is restricted to an area within 100 miles of Wiluna.

It seems probable that the development of the high liver copper levels was primarily due to the ingestion of herbage of high copper content. Data from the agricultural areas of Western Australia (Beck 1941, 1962) indicate that in those regions, levels of copper in pastures

TABLE I

Copper Content of Wiluna Sheep Livers

Values as p.p.m. Cu on dry liver

Property	Date	No. of Samples	Liver Cu		Details
			Mean and Range	Percentage of values above 1000 p.p.m.	
" Albion Downs "	Sept.-Nov., 1952	7	1010 300–1760	43	One sheep died Cu poisoning
	Jan.-April, 1953	6	1410 380–1990	83	Aged wethers
	July, 1953	2	860 780 940	0	
	Sept., 1953	3	1600 1190–2100	100	Aged ewes
	Sept., 1953	7	520 440 600	0	Young sheep 12–30 months
	Aug.-Sept., 1954	8	810 360–1390	37	
	Sept., 1955	12	860 390 1940	33	Mixed age ewes and wethers
	Sept.-Oct., 1956	6	1250 300 2140	83	Aged wethers
	May-Oct., 1959	6	620 220–1440	17	Aged ewes and wethers
" Yeelerrie "	Jan., 1954 .	6	1820 790–2730	67	Wethers mixed ages
	Feb.-Aug., 1955	8	780 210 1900	25	Wethers 3–6 years
" Lorna Glen "	March-April, 1954	5	1220 520–1850	60	Aged sheep
" Lake Way "	Nov., 1951 Feb., 1952	3	870 310–1750	33	
	Oct.-Nov., 1952 ...	6	720 350–2000	17	
	Jan.-March, 1953 . .	6	800 450–1380	33	Wethers 3–4 years
" Wanganoo "	July-Aug., 1956	6	450 180–740	0	Wethers 3–7 years
" Granite Peaks " ..	Aug.-Nov., 1952	6	380 150–820	0	Wethers 3–5 years
" Cunyu "	Aug.-Sept., 1956	6	300 140–520	0	Wethers 3–5 years
" Polele "	Oct.-Nov., 1952	3	290 210–440	0	Wethers 4 years
" Weebo "	Aug.-Sept., 1954	6	290 100–830	0	

rarely exceed 10-12 p.p.m. (dry basis). Dick (1954) has shown that cross-bred sheep will store dangerous amounts of copper when receiving more than about 10 p.p.m. copper in the diet, although merinos can, apparently, tolerate somewhat higher levels. The copper content of *Goodenia Mueckeana* was consistently high (14-22 p.p.m) and other species, at least in certain seasons, supplied amounts of copper well above normal. Seasonal variations in liver copper levels could be explained by the preferential grazing of species of lower copper content in some years.

The storage of copper in sheep is also controlled by factors other than the copper intake. Molybdenum above about 5 p.p.m. in the diet causes depression of copper storage provided adequate sulphate is present (Dick 1954). Very low molybdenum levels have been found in some of the species and this may have favoured copper storage. Sulphate levels were generally similar to those found elsewhere in Western Australia (Beck 1962). High levels have been observed in *Hibiscus pinonianus*, *Bassia* spp. and in three salt bushes, but the area of salt lake country was quite restricted on the properties where the investigations have been carried out. Similar high levels of sulphate have been found in halophytes elsewhere (Spais 1956; Barker 1961).

There is some evidence that manganese interferes with the limitation of copper storage imposed by molybdenum and sulphate (Anon. 1957-58) and it has been shown that very high levels of manganese cause an increase of copper storage in liver of the rat (Gubler *et al.* 1953). However, it is not known what effect the moderately high manganese levels of the Wiluna herbage would have on the copper storage of the grazing sheep.

Although the histological studies have given no definite evidence, it is still possible that hepatotoxic alkaloids may have contributed to the development of high copper levels in some instances.

The occurrence of liver copper levels up to 2700 p.p.m. in clinically healthy sheep indicates the very high concentrations which can be tolerated by sheep in the absence of stress. As it is well known that starvation will readily precipitate a fatal haemolytic crisis in such sheep, it is highly probable that some unexplained losses, reported during mustering and shearing, may have been due to copper poisoning.

Copper Poisoning in Sheep in the Toodyay Area.

In August, 1955, reports of heavy sheep losses were received from a 1,100 acres property some 10 miles north of Toodyay. A clinical diagnosis

of copper poisoning was confirmed by pathological examination and chemical analysis.

Copper fertilizers and supplements were not used and the sheep were bred on the property. The soils and pastures resembled those of a large belt of agricultural country where no cases of poisoning had been reported. At first it was thought that the poisoning might be similar to that encountered in sheep grazing on subterranean clover in certain seasons in Western Victoria (Anon. 1956). Analysis of the Toodyay pastures showed moderately high copper levels but the liver histopathology was quite distinct and suggested that a hepatotoxic plant was implicated (Bull, personal communication).

Geology and Soil Types

No geological survey of the area has been made but the rocks appear to be mainly biotitic gneisses and quartzite with dolerite intrusions.

The soils are red brown clay loams and sandy clay loams with non-calcareous subsoils.

History

The history was not very satisfactory. Losses from what was apparently copper poisoning were reported to have occurred on this property for many years. Young sheep were not affected. Losses were seasonal and occurred usually in August and September. In 1955, losses began earlier and the mortality was reported to be 50 in a flock of 1,700 Corriedale sheep. In 1956 there were no losses, in 1957 one or two cases and none in subsequent years. Occasional deaths from what appeared to be a similar condition have been reported from elsewhere in the district, but investigations have been confined to the one property.

TABLE II

Inorganic Constituents of Wiluna Herbage

Values expressed as mean and range on dry matter basis

Figures in brackets indicate number of samples analysed when less than the total number

Species	Date	Stations* Sampled	No. of Samples	Cu p.p.m.	Mo p.p.m.	Mn p.p.m.	Fe p.p.m.	SO_4 %
Goodenia Mueckeana	July, 1953	a c	4	19·9 17·1–20·9	< 0·1	900 790–1100	....	0·15 (2) 0·14–0·16
	Feb.-Aug., 1955	a b	4	19·1 13·8–21·7	< 0·1	760 570–1000	190 (2) 150–230	0·11 0·08 0·18
G. eremophila	Oct., 1952	a	1	15·7	...	...	....	...
	July, 1953	a	3	11·8 10·0–13·3	< 0·1 < 0·1–0·16	430 340–500	...	...
	Sept., 1954	a	1	6·8	< 0·1	490	.	0·36
	Aug., 1955	a b	2	8·3 8·0–8·7	< 0·1	1100 810–1400	..	0·16 0·08 0·25
	Oct., 1959	a	1	7·9	0·11	370	..	0·14
Helichrysum Davenportii (Everlasting)	July, 1953	a	1	20·3	...	...	...	..
	Sept., 1954	a	1	15·3	< 0·1	...	...	0·53
	Aug., 1955	a b	2	11·8 11·2–12·4	0·12 0·1–0·20	390 280–490	160 (1)	0·40 0·36–0·43
Hibiscus pinonianus	July, 1953	a b	4	14·6 12·3–16·5	0·27 (3) 0·15–0·47	92 (2) 82–103	....	1·2 (3) 0·9–1·5
	Aug., 1955	a b	2	9·4 7·3–11·6	0·38 0·22–0·55	97 95–100	225 (1)	0·62 0·51–0·74
Didiscus glaucifolius	July, 1953	a	1	13·6	< 0·1	...	...	....
Trichinium obovatum (Cotton bush)	July, 1953	a	1	13·5	0·28	100	...	...
	Aug., 1955	a b	2	5·6 5·5–5·8	0·72 0·31–1·13	57 50–65	165 (1)	0·16 0·15–0·17
T. exaltatum	July, 1953	c	1	10·1	0·86	...	...	...
Myriocephalus Guerinae (Billy button)	July, 1953	a	2	12·7 10·8–14·6	0·28 (1)	300 (1)	....	1·10 0·73–1·47
	Aug. 1955	a b	2	9·4 7·5–11·4	0·19 0·08–0·30	450 200–690	445 (1)	0·32 0·25–0·40
Eremophila leucophylla (Poverty or flannel bush)	July, 1953	a	4	12·3 9·8–13·9	0·15 (3) 0·13–0·17	600 400–740	..	0·21 0·15–0·27
	Aug., 1955	a b	3	12·8 10·3–14·5	0·22 0·13–0·29	450 190–870	160 (2) 150–170	0·26 0·22–0·30
Erodium cygnorum	July, 1953	a c	6	11·1 9·3 14·3	0·17 (5) < 0·1–0·37	130 (1)	....	0·34 (2) 0·24–0·44
Kennedya sp.	July, 1953	a	1	10·3	0·33	...	...	0·14
Sida corrugata	July, 1953	a	1	9·8	...	...	...	0·24
Danthonia bipartita (Wandarrie grass)	July, 1953	a	4	9·6 7·7–10·4	0·15 (3) 0·10–0·25	290 (2) 250–330	....	0·31 (2) 0·30–0·32
Ruelingia loxophylla	July, 1953	a c	3	9·0 6·9–11·0	...	275 (1)	..	0·12 0·08–0·15
Halorrhagis sp.	Aug., 1955	a	1	8·8	< 0·1	2400	207	0·37
Bassia spp.	July, 1953	a c	5	8·6 7·3–9·8	0·42 (2) 0·27–0·58	....	....	3·3 (4) 0·8–6·0
Codonocarpus cotinifolius (Native poplar)	July, 1953	a	2	8·6 7·9–9·3	0·62 (1)	360 (1)	...	0·79 (1)
Brachysema Chambersii (flowers only)	July, 1953	a	2	6·3 6·0–6·7	...	...	....	..
Acacia genistoides	July, 1953	a	1	3·3	..	...	....	0·10
Salt Bushes								
Kochia pyramidata	July, 1953	a	1	8·2	....	...	....	5·0
Atriplex paludosa	July, 1953	a	1	4·9	...	...	....	1·0
Lycium australe	July, 1953	a	1	9·3	....	...	...	2·0

* "a" indicates "Albion Downs," "b" "Yeelirrie" and "c" "Lorna Glen."

TABLE III

Copper, Molybdenum and Inorganic Sulphate Levels of Toodyay Pastures

Values expressed as mean and range on dry matter basis

Date	No. of Samples	Details	Cu p.p.m.	Mo p.p.m.	SO_4 %
Oct., 1955	2	Capeweed	14·4	0·65	0·30
			14·2–14·6	0·60–0·70	0·28–0·32
	1	Sub. clover	14·6	0·40	0·24
	1	Mixed sub. clover and capeweed	9·7	0·50	0·33
Aug., 1956	6	Grasses	6·7	1·37	0·19
			4·3–8·6	0·62–2·26	0·13–0·34
	4	Capeweed	6·7	1·87	0·19
			5·4–8·3	1·6–2·6	0·17–0·22
	2	Mixed pasture	9·7	1·10	0·17
			9·2–10·2	0·95–1·25	
Sept., 1956	1	Capeweed	14·4	0·49	0·19
	1	*Plantago cretica*	9·2	0·49	0·28
	1	Mixed pasture	11·0	0·60	0·18
Aug., 1957	4	Capeweed	11·5	0·63	0·28
			9·1–14·0	0·48–0·94	0·17–0·49
	1	Grasses	8·4	0·61	0·17
	2	Sub. clover	12·0	0·16	0·12
			10·2–13·9	0·14–0·18	0·07–0·18

Pasture Investigations

The pastures were of the annual Mediterranean type common to the 20-25 inch rainfall belt. The main species were *Trifolium subterraneum*, capeweed *(Cryptostemma calendula)*, wild geranium *(Erodium botrys)* and annual grasses *(Bromus* spp. *Vulpia myuros* and Wimmera rye grass, *Lolium* spp.). Surveys were carried cut by the former Government Botanist (Mr. C. A. Gardner) in August, 1955, and September, 1956, but the only unusual species found was *Plantago cretica.* An examination of dry paddocks in March, 1957, showed no unusual plants and none considered likely to cause toxic effects. *Echium plantagineum* ("Pattersons curse") and lupins *(L. varius* and *L. angustifolius)*, known to cause liver damage, were completely absent from the property.

Analysis of pasture samples is set out in Table III. Levels of molybdenum and inorganic sulphate were determined as these are known to affect copper metabolism. Two samples (August, 1956) were analysed for manganese content but normal levels were found (72 and 85 p.p.m. on dry matter).

As the histology described in the following section had suggested the action of a hepatotoxic alkaloid, determinations for alkaloid content were made on *P. cretica* at the C.S.I.R.O. Chemical Research Laboratories, Melbourne. As the manager of the property had stated that cases of poisoning only occurred in years of rank capeweed growth, this species was also examined, even though there was no suggestion that it caused trouble elsewhere. Both species showed very low alkaloid content *(P. cretica,* 0.018 per cent. tertiary base, quaternary and weak bases and N oxides absent; capeweed, 0.014 per cent. tertiary base and 0.004 per cent. N oxide).

Animal Studies

Chemical.—The liver of the sheep dying of copper poisoning in August, 1955, showed 900 p.p.m. copper (dry basis); a similar sheep in July, 1957, showed 600 p.p.m. These values are rather lower than those usually found in cases of haemolytic jaundice due to copper poisoning.

In June, 1956, liver samples for chemical and histological examination were collected at the abattoirs from 30 sheep from the property. The sheep had been without food for at least 18 hours and consequently the livers contained more fat than usual. There had been no losses from haemolytic jaundice during yarding. Some of the livers were macroscopically abnormal; two were small, five were yellowish and one rather fibrous. Chemical analysis showed the following results which are expressed as the means with range of values in parenthesis; the values for fat are on the dry material and values for iron and copper on the dry, fat-free material:—copper 1500 p.p.m. (490-3120), iron 990 p.p.m. (280-2410), fat 23 p.c. (15-40). Seventy-three per cent. of the livers contained over 1000 p.p.m. copper. Livers from normal Western Australian sheep contain 50-400 p.p.m. copper, 200-800 p.p.m. iron and less than 10 per cent. fat.

Histology.—Sections were available from the two moribund animals and the thirty abattoirs animals mentioned above.

In the liver from the first affected animal (August, 1955) the interstitial tissue in the portal tracts was slightly increased and abnormally cellular. A slight fine diffuse fibrosis was present. Excess bile pigment was present in the ducts and canaliculi. There was some new bile duct formation. Ceroid and protein inclusion globules were plentiful. but megalocytosis and central necrosis were absent. The kidney tubules were laden with haemoglobin casts and degradation products. The second liver (July, 1957) showed marked portal tract fibrosis infiltrated with lymphocytes and polymorphs. These reactive cells were also significantly increased in numbers, both diffusely and focally throughout the liver, presumably as a reaction to necrosis of liver cells. There was a great variation in nuclear size. The reticuloendothelial cells were increased in numbers, swollen and packed with degenerating red blood cells and bilirubin. There was some small bile duct proliferation in the portal tracts.

The livers of many of the abattoirs sheep showed cellular reaction in the portal tracts

with fibrosis and bile duct damage. The Kupffer cells frequently showed yellow-brown granules.

Discussion

Copper levels of pasture were at times moderately high but it is not considered that these alone could have caused a dangerous accumulation of copper in sheep livers. Molybdenum and inorganic sulphate levels were normal.

The histological data on livers were limited but suggested that two processes were involved. The first consisted of fibrotic and bile duct changes probably leading to some degree of excretory obstruction. This had probably been acting for some time before deaths occurred, and could have been due to ingestion of a plant containing a hepatotoxic alkaloid or to recovered facial eczema due to fungal toxicity. The absence of megalocytosis indicated a differnt type of poisoning from that due to *Heliotropium* (Bull 1961). The second change in the livers was due to an acute haemolytic process which caused the actual deaths. Although the clinical findings indicated that this was due to copper poisoning, the histological picture gave some suggestion that it may have been due to difficulty in the excretion of bilirubin normally produced. The relatively low liver copper liver values for the two sheep which died also gave some support to the idea that copper toxicity was not the primary cause of death.

It is not possible to give a satisfactory explanation for the massive accumulation of copper in the thirty abattoirs sheep but it was probably consequent on the liver damage as in heliotrope and lupin poisoning.

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